

COMMUNICATIONS

Detection of Anti-*Amyloodinium ocellatum* Antibody from Cultured Hybrid Striped Bass (*Morone saxatilis* × *M. chrysops*) during an Epizootic of Amyloodiniosis

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Abstract.—After an epizootic of amyloodiniosis (caused by the protozoan *Amyloodinium ocellatum*) in a commercial aquaculture facility for hybrid striped bass (female striped bass *Morone saxatilis* × male white bass *M. chrysops*), sera from these fish, as well as from others that had been experimentally immunized with the parasite, were evaluated by an enzyme-linked immunosorbent assay for antibody specific for the parasite. Titers were similar between the fish infested in culture and the experimentally immunized fish, and were significantly higher in both the cultured and artificially exposed fish than in unexposed fish. These results suggest that an infestation of *A. ocellatum* can stimulate the production of humoral antibodies to the parasite, providing further evidence that natural infestations of the parasite may confer protective resistance in fish that survive the initial parasitic infestation.

Amyloodinium ocellatum is a ubiquitous, euryhaline dinoflagellate that is capable of infesting a wide range of estuarine and marine species of fish (Sindermann 1990). The organism has been responsible for numerous epidemics in aquaculture facilities throughout the world (Ghittino et al. 1980; Paperna 1980; Baticados and Quintino 1984). Despite the importance of *A. ocellatum* in both wild and mariculture species of fish, relatively little is known of the immune response and protective mechanisms by which a host may resist an infestation. Lawler (1980) and Paperna (1980) have reported that some species of fish acquire resistance after a sublethal exposure to *A. ocellatum*. A particularly severe epizootic of amyloodiniosis in pond-cultured juvenile hybrid striped

bass (female striped bass *Morone saxatilis* × male white bass *M. chrysops*) in a commercial aquaculture facility in North Carolina (Noga et al. 1991) provided the opportunity to evaluate sera from these fish by an enzyme-linked immunosorbent assay (ELISA) for the presence of antibody specific for the parasite.

Methods

Eleven juvenile hybrid striped bass were presented to the Aquatic Medicine Laboratory at the College of Veterinary Medicine at North Carolina State University for diagnostic evaluation. After a definitive diagnosis of amyloodiniosis was made by microscopic examination of gill, skin, and fin samples from several fish, blood was collected from the caudal tail vessels of five morbid individuals. The blood was allowed to clot at room temperature for 1 h, then the serum was separated by centrifugation at 14,000 × gravity for 4 min in pediatric centrifuge tubes (Microtainer, Becton Dickinson and Co., Rutherford, New Jersey) and stored at -70°C. Six weeks later, seven additional fish that had survived the *A. ocellatum* infestation were obtained from the same pond, and serum was collected and stored as described above.

In addition, 27 juvenile hybrid striped bass were obtained from stock maintained at the laboratory. The fish were housed in a 1,100-L recirculation system at 0‰ salinity and fed a commercial pelleted diet (Zeigler Brothers, Inc., Gardners, Penn-

TABLE 1.—Optical density values and ELISA values (EV) for the four groups of hybrid striped bass tested for anti-*Amyloodinium ocellatum* antibody.

Fish group	N	Optical density			Mean EV
		Mean	Range	SD	
Control (nonexposed)	15	0.0336	0.0000–0.0605	0.021	<1
Experimentally immunized	12	0.1993	0.0440–0.4053	0.114	59.3
Acute epizootic	5	0.2383	0.2008–0.3063	0.039	70.0
Six weeks postepizootic	7	0.1876	0.1633–0.2118	0.018	48.8

sylvania). Twelve of these fish were selected, tagged through the dorsal musculature, and immunized intraperitoneally with 0.5 mL of parasite culture media containing 1×10^5 live dinospores of *A. ocellatum* as described by Smith et al. (1993). The immunized fish were then maintained in a separate 450-L recirculation system. The remaining 15 fish were immunized intraperitoneally with 0.5 mL of culture media without dinospores and served as negative controls for the ELISA. Blood was collected from all fish 15 d postimmunization, and the serum was collected and stored as described above.

Production of rabbit anti-hybrid striped bass antibody followed the technique of Smith et al. (1993). Briefly, hybrid striped bass immunoglobulin specific for a particular antigen was affinity-purified with agarose beads to which the antigen was covalently bound. A laboratory rabbit was immunized with the purified hybrid striped bass immunoglobulin and hyperimmune rabbit anti-hybrid striped bass serum collected after an appropriate time interval.

The ELISA technique for detecting antibodies to *A. ocellatum* was modified from Smith et al. (1992). The techniques were essentially the same as those previously reported for blue tilapia *Tilapia aurea*, except that rabbit anti-hybrid striped bass serum (1:1,000 dilution) was used in the assay instead of rabbit anti-tilapia serum. Antibody titers were expressed as a standardized ELISA value (EV), calculated from mean optical density (OD) values of quadruplicate wells as described by Horwitzky and Searson (1986). An EV greater than 2 was considered positive (de Savigny and Voller 1980).

Results

After evaluation of the control fish with no known exposure to *A. ocellatum*, the minimum positive OD value (Table 1) was set at a mean OD value of 0.0336 for nonexposed individuals plus three standard deviations ($1 \text{ SE} = 0.021$) of the mean (de Savigny and Voller 1980; Kodoma et al.

1985). Thus, for this ELISA an OD value greater than 0.0966 was considered positive for anti-*A. ocellatum* antibody. The mean OD values for wells containing serum from fish immunized with antigens of the parasite was 0.1993. Therefore, the mean EV for the hybrid striped bass immunized with live dinospores of *A. ocellatum* was 59.3. Serum from hybrid striped bass sampled during the acute stage of the infestation had higher mean OD values and EVs. Surviving hybrid striped bass taken from the same pond 6 weeks later had mean serum values lower than those of either exposed group (Table 1).

Discussion

Amyloodinium ocellatum is one of the most pathogenic parasites of warmwater marine species of fish (Sindermann 1990). Epizootics caused by this organism have resulted in morbidity and mortality in numerous mariculture facilities, and the occurrence of *A. ocellatum* is likely to become more prevalent as intensive aquaculture expands. Previous studies with *A. ocellatum* have shown that fish experimentally immunized with antigens of the parasite develop a humoral immune response (Smith et al. 1992). It was also shown that blue tilapia immunized with live dinospores of the parasites developed a significantly greater humoral immune response than those immunized with antigens of the sonicated parasite (Smith et al. 1993). As a positive control for the present investigation, hybrid striped bass were immunized intraperitoneally with live dinospores obtained from cell culture. As expected, the immunized hybrid striped bass developed a substantial, titerable humoral immune response.

Cultured hybrid striped bass infested with *A. ocellatum* also developed a titerable humoral immune response. Because the length of time that the parasite was present on the fish prior to the onset of clinical signs was unknown, the humoral immune response as indicated by the EVs of the experimentally immunized fish and those infested in culture cannot be directly compared. However,

it can be assumed that the cultured fish had been infested for some period of time, because the parasite would presumably have to proceed through numerous generations to reach a level sufficiently pathogenic to cause mortality in the hybrid striped bass. And, although the acute and postinfestation values were not obtained from the same individual fish, the values were obtained from the same population of fish maintained in the pond in which the amyloodiniosis had occurred. Therefore, as would be expected, the mean anti-*A. ocellatum* serum titer of the hybrid striped bass population had declined over the 6-week period following successful treatment of the parasitic infestation.

The results of this study indicate that in hybrid striped bass, an epizootic in culture with *A. ocellatum* stimulates the production of a specific humoral immune response. This would suggest that a persistent natural infestation, whether a constant or recurring infestation, could continue to stimulate or potentiate an already existing humoral immune response.

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